AFTER-RIPENING AND GERMINATION OF JUNIPERUS SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 275

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(WITH ONE FIGURE)

Some seeds fail to germinate in compensating percentages or even at all when placed under ordinary germination conditions. Because of inquiries directed to this laboratory from various growers concerning the best methods of handling juniper seeds, there was conducted a careful study of after-ripening, germination, and seedling development, as well as some of the chemical and physiological changes involved in these processes. Strict quarantine laws, recently put into effect, will mean that many species of decorative plants that were formerly grown from seeds in foreign countries and brought to America as plants, must now be grown from seeds by American nurserymen. This will doubtless promote study of the germinative behavior of many refractory seeds in the future.

Literature

Wild plants of the temperate zone produce seeds that usually have a rest period, which varies as to length and cause with the different species and kinds of seeds. This dormancy is found to be characteristic of the seeds of 75 per cent of the wild and the cultivated plants studied by HOWARD (18). Although the rest period of most seeds is only a few months, it may be years, as in the case of some Conifers (21) and of *Euphorbia Cyparissias* (19). CROCKER (5) states that delayed germination is due to one or more of the following conditions: (1) rudimentary embryo, (2) dormant embryo, (3) coats inhibiting embryo expansion, (4) coats inhibiting gas exchange, (5) coats inhibiting water absorption, (6) a combination of two or more of these, and (7) secondary dormancy. Up to date seeds have been studied that represent each of these different types of dormancy.

As it has been impossible to dispense with this rest period in all cases, many substances have been used to reduce dormancy and force seeds to germinate. Concentrated sulphuric acid has been used by HILTNER and KINZEL (17), ROSTRUP (32), and others with positive results. Among the salts ROSE (31) noted that the sulphates and nitrates were the better forcing agents. Hydrogen peroxide and increased oxygen pressure forced the germination of *Xanthium* seeds (5). Wounding and treatment with ether stimulated the germination process (3). Light has been found to force or to inhibit germination depending on the seed (12, 20). The New York Experiment Station (24) and many others have shown that desiccation improves the germinating power of corn. The hot bath has been used with success on some seeds (4). Alternating temperatures have been used to force grass seeds in the Seed-testing Laboratories of the Bureau of Plant Industry. With these much has been claimed for freezing and thawing as a forcing agent (29). LAKON (21), however, found that the germination of *Pinus Peuce, P. Cembra, P. Strobus*, and *P. silvestris* could not be accelerated by treatment with dry heat, warm bath, file injury, ether, chloroform, salt solutions, concentrated sulphuric, or dilute acids.

Seeds with dormant embryos must go through a series of changes (after-ripening) before germination can occur (5). The after-ripening of hawthorn seeds proceeds fastest at 5–6° C. according to DAVIS and ROSE (8). An idea of this after-ripening process may be gained by following the results of LAKON on a protein and ECKERSON (10) on a fatty seed. LAKON (22), in studying the changes that precede germination of *Fraxinus excelsior*, found very little increase in water absorption. From the tenth day on, starch accumulated in the embryo cells, with a corresponding disappearance of protein from the endosperm cells. In place of the disappearing protein a turbid emulsion formed, which later was digested. At no time did starch appear in these endosperm cells. The embryo doubled its length during this process of "Vorkeimung." ECKERSON (10) studied the changes occurring in the hawthorn seed during after-ripening, and reported an increasing acidity and water absorbing power of the dormant organ. The catalase, peroxidase, and oxidase activity increased as after-ripening and

germination proceeded. Germination was accompanied by a decrease of stored fats and an increase of sugar. Although the details varied somewhat, both seeds passed through a period of preparation for germination.

Material and preliminary study

The *Juniperus* plants are erect or prostrate dioecious Cupresseae distributed over the Northern Hemisphere. They are used in landscape decoration, serving as hedges and screens up to 30 ft. high. In early spring the flowers appear in the leaf axils, forming many carpel whorls, of which only the upper one develops. This whorl bears 3 ovules, which grow together and form a spherical fruit, which requires two years to ripen, and contains 1-3 seeds.

TABLE I

MATERIAL SECURED

Species	Lot	Date	Place
J. virginiana L.	1	November 11, 1918	West Newberry, Massachusetts
J. c. depressa Pursh.	2	January 1, 1919	Boxford, Massachusetts
J. communis L.	3	January 1, 1919	Vermont
J. prostrata Pres.	4	January 1, 1919	Vermont
J. virginiana L.	5	January 1, 1919	Vermont
J. communis L.	6	April 19, 1919	Near Chicago, Illinois
J. virginiana L.	7	April 19, 1919	Near Chicago, Illinois
J. communis L.	8	September 19, 1919	Near Chicago, Illinois
J. virginana L.	9	September 19, 1919	Near Chicago, Illinois

Juniperus seeds were gathered in the fruit condition, and those used in these experiments were collected as stated in table I.

The seeds freed from the fruit vary with the species as to color, shape, size, and quality. Those of *J. virginiana* are light brown' smooth, brittle, 3-4 mm. long, and when air-dry weigh about 0.009 gm. each. Seeds of *J. c. depressa*, *J. communis*, and *J. prostrata* are much alike. These seeds are dark amber, rough, 4-6 mm. long, narrower and less brittle than those of *J. virginiana*. Some of the *J. virginiana* material proved to be badly worm eaten, while the other lots were quite free from worms. Seeds collected in Vermont were generally good. Table II gives the percentage of bad seeds due to worms and lack of development.

Fig. 1 shows the structure of the seed of *J. virginiana*, with its many membranes and protective layers. In strong contrast with the hard brown coat are the clear white endosperm and embryo. The hard coat consists of three layers: the outer fleshy (*a*), the stony (*b*), and the heavy inner fleshy (*c*). In the outer fleshy layer are found pectic substances and methyl pentosans. The stony layer is lignified and contains other substances, as calcium, pectates, and pentoses. The inner fleshy layer is well developed and consists of suberin with some little cellulose. Of the endosperm, embryo, etc., one distinguishes the nucellus (*d*), the mass of distorted tissue (*e*), the hypocotyl cap (*f*), the megaspore membrane (*j*), the endosperm wall (*k*), the endosperm (*g*), and the embryo (*h*).

TABLE II

PERCENTAGE OF IMPERFECT SEEDS IN LOTS 1, 3, 4, AND 5

Species	Lot	No. examined	Percentage imperfect
J. virginiana	1	100	59
J. virginiana	1	50	63
J. virginiana	1	100	61
J. virginiana	1	2000	60
J. communis	3	1000	26
J. prostrata	4	1000	20
J. virginiana	5	2000	22.5

The nucellus is constructed of long narrow cells which give tests for cellulose and pectic acid. The mass of tissue (*e*) protecting the hypocotyl consists of cellulose, pectic substances, and some other groups of substances such as fats and gums. Between this mass and the hypocotyl is a cap of very fine and firm cells (*i*), which are made up of cellulose and hemicellulose. The megaspore membrane (*j*) is very thin and stains with ruthenium red. Examination shows that the outer wall of the outer layer of endosperm cells has been developed into a suberin wall (*k*). This wall is insoluble in concentrated H_2SO_4, 50 per cent chromic acid, and gives the phellic acid reaction. The endosperm cell walls are rather thick and made up of cellulose. Cell walls of the embryo are thin and consist of pectose and cellulose.

The storage substances of the resting seed are mentioned here, but they are later taken up in detail with the changes accompanying germination. Tannin is generally distributed throughout the coat. Some is stored in the nucellus, endosperm wall, and hypocotyl cap, but none was found in the endosperm or embryo. The embryo and endosperm are stored with an abundance of protein and fat, and also a trace of glucose. No starch was found in either endosperm or embryo. Histidine, tyrosine, and arginine are found in both endosperm and embryo. There is also a trace of leucine and probably cystine.

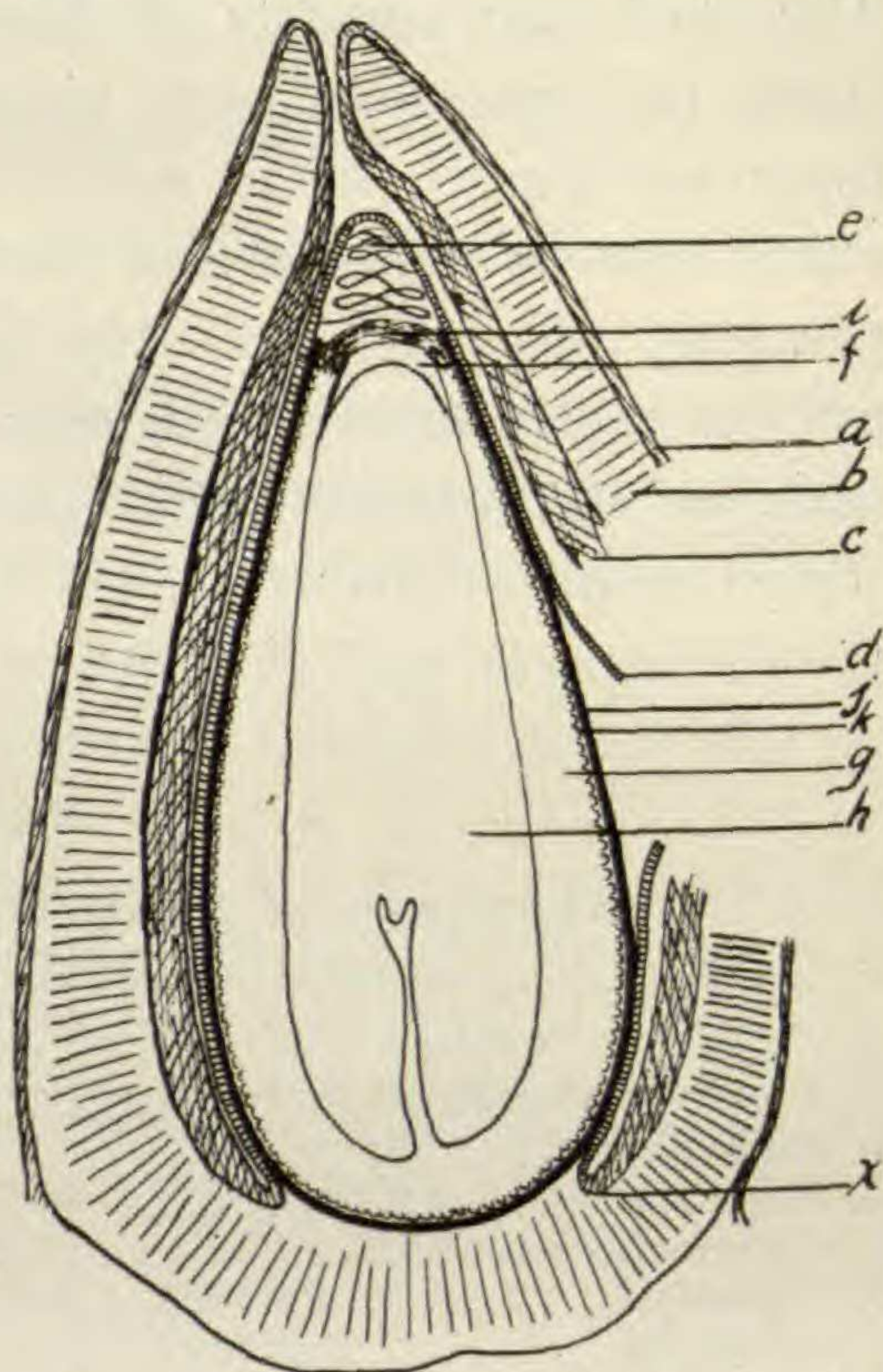

FIG. 1.—Longitudinal section of seed of *Juniperus virginiana* with part of nucellus and integument removed from one side: *a*, outer fleshy; *b*, stony; *c*, inner fleshy; *d*, nucellus; *e*, distorted tissue; *f*, hypocotyl cap; *i*, protective cap; *j*, megaspore membrane; *k*, endosperm wall; *g*, endosperm; *h*, embryo.

Catalase activity of the embryo and endosperm is low, while that of the coat is negligible. The seed shows peroxidase activity, with a mere trace of oxidase activity.

The resting seed embryo has a P_H value of about 8, while the endosperm has a P_H value of about 5. Thus the embryo is basic, while the endosperm is acid, a condition opposite to that usually found in seeds which are ready for germination.

Treatment of material

After collection the larger part of the fruit or berry was removed from the seed by running the berries through a coffee mill so wide open as not to injure the seed. Next the seeds were sifted and the milling and sifting repeated. The seed material was then rubbed between two sieves in the presence of an abundance of water. In this way all the berry and excess tissues which prevent

sterilization were easily and quickly removed. The bad seeds were floated off with water, and the good seeds rinsed and permitted to dry before sterilization.

After some testing, a 5 per cent solution of formalin acting for 2.5 minutes was selected as the best sterilizing agent for juniper seeds. It was found that formalin did not readily penetrate the coat, reduce the catalase activity, or hinder germination. The permeability of the coat was studied as follows. Seeds were submerged in different solutions for a definite time, removed, washed in distilled water, the coats removed, the seeds sectioned with a freezing microtome, and the sections tested for the respective solutions. Table III shows that the coat was very permeable to water, bases, and salts, but not permeable to stains and acids.

TABLE III

PERMEABILITY OF COATS TO WATER, STAINS, ACIDS, BASES, FORMALIN, AND SALTS

Substance	Permeability	Substance	Permeability
Eosin (dilute)	Impermeable	Water	Very permeable
Eosin (strong)	Impermeable	C_2H_5OH	Very permeable
Neutral red (dilute)	Impermeable	NaOH	Very permeable
Neutral red (strong)	Impermeable	KOH	Permeable
Formalin	Slowly permeable	NH_4OH	Permeable
HCl N/100	Very impermeable	$AgNO_3$	Very permeable
H_2SO_4 N/100	Very impermeable	$HgCl_2$	Very permeable

That the salts ($AgNO_3$ and $HgCl_2$) penetrated the coats is shown by the catalase activity of the seeds with coats removed (table IV). These seeds, after being sterilized, washed, and incubated at 9° C. for 48 hours, had coats removed, and were ground for catalase activity determinations. It was further shown that $AgNO_3$ penetrated the coats by the fact that seeds so treated were killed. Seeds sterilized in formalin germinated, and therefore most of the seeds used in these experiments were sterilized 2.5 minutes in 5 per cent formalin. In this connection it should be noted that SCHROEDER (33) and GROVES (14) found that the coat of the wheat seed was practically impermeable to $AgNO_3$, and that this solution was a good sterilizing agent for wheat. This shows how the permeability of seed coats may vary with different seeds.

For some experiments the seeds were freed from the probable inhibiting influence of the hard coats by one of the three following treatments: (1) dry seeds were put into concentrated H_2SO_4 and the rate of penetration followed by testing with congo red; it required 24 hours to entirely carbonize the coat; the carbonized coats were rubbed off with filter paper and the seeds rinsed in a suspension of $CaCO_3$ and distilled water; (2) seed coats were also removed with seed nippers; (3) in other experiments only the end of the coat was cut away. The sterile seeds were put into sterile wide mouthed bottles, Petri dishes, or flasks for germination. Those cultures

TABLE IV

EFFECT OF STERILIZING AGENTS ON CATALASE ACTIVITY OF SEEDS, TREATED 2 MINUTES (40 SEED COATS REMOVED)

Sterilizing agents	Oxygen in cc. liberated during		
	1 min.	5 min.	10 min.
Water (check)	9.0	20.0	24.3
Water (check)	8.9	19.0	24.0
Formalin 5 per cent	8.5	21.0	24.8
Formalin 5 per cent	8.8	21.2	24.4
$AgNO_3$ 2 per cent	3.0	6.0	8.0
$AgNO_3$ 2 per cent	3.1	6.4	8.1
$HgCl_2$ 1 per cent	4.0	11.2	13.0
$HgCl_2$ 1 per cent	4.1	10.0	11.8

which required good ventilation were protected against infection by a system of tubes plugged with cotton. The seeds were left on the moist walls of the containers or on moist filter paper, depending upon the conditions of the experiments. In the determination of the effect of solutions as forcing agents, no foreign absorbing material was allowed in the flasks with the seeds. In all other cases, except where mentioned, the seeds were placed on moist filter paper.

Forcing agents

The change of the catalase activity and the ability of the seeds to germinate were used as standards to determine whether or not the substance or treatment under examination was a forcing agent for the juniper seed.

METHODS.—As it has been shown that catalase activity increases with after-ripening of the dormant-embryo seeds (**6, 10**), catalase activity was chosen as the first standard. Germination, the production of independent seedlings, was selected as the final standard. Great care was found necessary in the preparation of material and the manipulation of the catalase apparatus. As the berry has a high catalase activity, every trace of fruit was removed before grinding. The grinding was carried out under similar conditions, and with a definite amount of water and no. 2 sand per unit weight of seed material. The dioxogen was neutralized with N/10 NaOH at the time of using. All determinations were made with the bath at 25° C., and the drive wheel of the apparatus regulated to make 30 revolutions per 10 seconds. No explanation is needed for germination as a standard.

FORCING AGENTS.—Among the common forcing agents tried on the juniper seeds were high temperatures, alternating temperatures, removal of coats, hydrogen peroxide, dilute acids, carbon dioxide, light, soil, mercuric chloride, ether, and oxygen. The first seven of these had very little effect on the catalase activity and did not force germination. While the treatments with mercuric chloride, ether, and oxygen did not force germination, each had its influence upon catalase activity.

CROCKER and HARRINGTON, in an unpublished work at the Seed-testing Laboratories of the Bureau of Plant Industry, have found that $HgCl_2$ was a good forcing agent for Johnson grass. Juniper seeds were sterilized and put into flasks containing the following concentrations of $HgCl_2$. After 24 hours the excess of liquid was poured off. The results are given in table V. These data, as well as those obtained by grinding the seeds for catalase activity in the same concentrations, show that $HgCl_2$ reduced catalase activity in the higher concentrations. None of the seeds treated with $HgCl_2$ germinated.

In studying the effect of ether, seeds were sterilized, put into Petri dishes without covers, and exposed to air containing various amounts of ether by sealing in 9 liter cans. After a certain exposure the seeds were removed, aired, and placed to germinate. Table VI gives the catalase activity for seeds that were exposed to ether

6 days and then left in the germinator 95 days at 25° C. Seeds similarly treated, except that they were exposed only 2 days, gave less marked catalase activity. The point to be noted in table VI is the increased activity which was given by seeds treated with the larger amounts of ether. Of added interest is the fact that there

TABLE V

CATALASE ACTIVITY OF SEEDS TREATED 95 DAYS WITH $HgCl_2$ SOLUTIONS AND PERCENTAGE OF GERMINATION AFTER 6 MONTHS

CONCENTRATION OF $HgCl_2$ USED	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
N/800	0.5	2.3	2.9
N/1600	1.6	3.0	3.4
N/3200	1.3	3.1	3.9
N/6400	2.5	3.5	4.0
N/12800	3.0	4.0	4.6
N/25600	3.0	4.5	5.0
N/51200	3.2	5.0	5.9
N/102400	2.9	5.5	6.2
Water (check)	2.8	5.5	6.0

TABLE VI

EFFECT OF ETHER ON CATALASE ACTIVITY OF JUNIPER SEEDS KEPT AT 25° C.

AMOUNT OF ETHER PER 9 LITERS OF AIR	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
1.4 cc.	2.9	6.0	6.1
2.8 cc.	3.5	7.8	8.4
5.6 cc.	4.8	10.0	10.9
8.4 cc.	5.0	10.5	11.0
16.8 cc.	5.6	11.0	11.5
Without ether (check)	2.8	5.5	6.0

was no germination. It is suggested, therefore, that one may have an enormous increase of catalase activity without a corresponding after-ripening of the dormant embryo.

To study the effect of oxygen, seeds were sterilized and exposed to air containing the following percentages of oxygen. Table VII gives the catalase activity for seeds at the end of 45 days. With the higher percentage of oxygen there was an increase of catalase

activity. At the forty-fifth day the remaining seeds were exposed to atmospheric air. Table VIII gives the catalase activity for the same lot of seeds at the end of 95 days, 50 days after replacing the oxygen by air. The point of interest here is the fall in catalase

TABLE VII

EFFECT OF OXYGEN ON CATALASE ACTIVITY OF SEEDS STORED 45 DAYS AT 25° C.

Percentage of oxygen	Lot	Oxygen in cc. liberated during		
		1 min.	5 min.	10 min.
30	1	2.5	4.4	4.8
55	2	3.4	5.3	5.6
80	3	3.7	6.2	6.6
100	4	4.8	7.5	8.8
Air (check)		3.8	6.2	6.9

TABLE VIII

REDUCED CATALASE ACTIVITY IN OXYGEN TREATED SEEDS WITH DECREASE IN PERCENTAGE OF OXYGEN

Lot	Oxygen in cc. liberated during		
	1 min.	5 min.	10 min.
1	2.8	5.0	6.0
2	2.9	5.1	6.1
3	2.7	5.0	5.8
4	2.8	5.0	5.6
Air (check)	2.8	5.5	6.0

activity, at the ninety-fifth day, for the seeds that were exposed to 100 per cent O_2 during the first 45 days. None of these seeds germinated.

TEMPERATURE.—No other condition affected the development of the juniper seeds to the extent that temperature did. Both alternating and constant temperatures ranging from 15–30° C. were found to reduce the catalase activity and inhibit germination. Seeds exposed to winter weather (in soil and on moist filter paper) gave about 1 per cent germination. Those subjected to a temperature of 10–12° C. in running water showed a steady increase of catalase activity up to the time of germination. Between the

fourth and sixth month the germination reached 10 per cent, a very marked increase over that obtained at the higher temperatures. When the temperature of the water rose much above 12° C. germination ceased. These results show that the increased germination was not due to the removal of inhibiting substances from the coat, but to the effect of the low constant temperature.

Although many observers (**11, 20, 29, 30**) have reported a forcing action for freezing and freezing with thawing, these results show that when freezing really occurs it is very injurious. On March 14, 1919, 1000 air-dry seeds and 1000 moist seeds were placed at a constant temperature of $-23°$ C.; and 1000 air-dry and 1000 moist seeds were subjected to an alternation of temperature between $-23°$ and 10° C. The latter seeds were left at each temperature for one week. After 45, 95, and 150 days of exposure samples were removed for study. The catalase activity of these seeds for 45 and 95 days is given in table IX. The catalase activity of seeds stored dry at $-23°$ C. equaled that of untreated seeds, while that of seeds stored wet at $-23°$ C. and wet or dry at the alternating temperature showed a marked decrease. There was no change in the oxidase or peroxidase activity. The seeds stored dry at $-23°$ C. showed no increase of H^+ ion or titratable acid over that of the untreated seed. All other seeds showed a slight increase of sugar content and of H^+ ion concentration; also a 40 per cent gain of titratable acid. Both embryo and endosperm of these seeds, stored at the alternating temperatures, had the same H^+ ion concentration. The fats in these seeds were very soluble, not characteristic, and diffused throughout the endosperm and embryo. This general diffusion of the fats and the equal H^+ ion concentration for the embryo and endosperm indicate that the membranes had become more permeable by freezing (**16**). On staining these seeds with methylene blue they appeared to be dead. Samples of all seeds were put under favorable conditions for after-ripening and germination, but only the seeds that were stored dry at $-23°$ C. after-ripened and germinated. These results prove that these low temperatures are very injurious unless the seeds are dry. It is probable that seeds stored at this low constant temperature and protected from moisture would retain their viability many years.

The alternation of temperature between $-23°$ and $10°$ C. kills moist *Juniperus* seeds very soon, and in no sense can be looked upon as a forcing agent.

One lot of moist seeds was subjected to a temperature of $-5°$ C., and a second lot to an alternating temperature of -5 and $5°$ C. The latter was exposed to each temperature for one week

TABLE IX

EFFECT OF TEMPERATURE ON CATALASE ACTIVITY OF JUNIPER SEEDS (NO. 10)

Material and treatment			O_2 in cc. liberated during			Total loss or gain over that of dry seeds (cc.)
Condition	Weight in gm.	Temperature °C.	1 min.	5 min.	10 min.	
	Stored 45 days					
Dry	0.025	....	2.6	5.4	5.8	....
Dry	0.025	−23	2.5	5.4	5.7	−0.1
Wet	0.026	−23	2.4	5.0	5.5	−0.3
Dry	0.023	−23 and +10*	2.2	4.9	5.4	−0.4
Wet	0.026	−23 and +10*	1.6	3.2	3.6	−2.2
Wet	0.025	− 5	3.0	5.1	5.8	0.0
Wet	0.025	0	3.0	6.0	6.5	+0.7
Wet	0.026	− 5 and + 5*	3.5	6.8	7.0	+1.2
Wet	0.026	+ 5	3.6	7.5	9.1	+3.3
Wet	0.026	+10	3.5	6.6	7.0	+1.2
Wet	0.026	+25	3.0	6.2	6.9	+1.1
	Stored 95 days					
Dry	0.023	−23	2.3	5.5	5.8	0.0
Wet	0.023	−23	2.4	5.1	5.7	−0.1
Dry	0.026	−23 and +10*	2.0	4.2	5.0	−0.8
Wet	0.025	−23 and +10*	1.0	2.8	3.1	−2.7
Wet	0.025	− 5	3.0	6.4	6.5	+0.7
Wet	0.025	0	3.4	7.0	8.0	+2.2
Wet	0.026	− 5 and + 5*	3.8	8.0	9.0	+3.2
Wet	0.026	+ 5	5.0	9.1	12.0	+6.2
Wet	0.026	+10	3.9	8.2	9.2	+3.4
Wet	0.026	+25	2.8	5.5	6.0	+0.2

*Weekly alternated between the two temperatures.

at a time. Table IX shows the catalase activity of these seeds for 45 and 95 days exposure. The lot stored at $-5°$ C. showed scarcely any increase of catalase activity, while the lot exposed to the alternating temperature was more active. Both lots appeared morphologically and physiologically in good condition. There was a slight accumulation of sugar in all seeds. The first showed

no germination, but some of the latter germinated after about 6 months. While exposure to $-5°$ C. was not sufficient to injure the ungerminated seeds, it proved fatal to the germinated seeds. This is due to the fact that when the coat splits open the endosperm and embryo just doubles its water content and thereby dilutes the cell sap to a degree which permits ice crystals to form. Seeds at this period and later periods of development were killed by exposure to $-5°$ C. for seven days or less. The after-ripening and gain in catalase activity was a little more than enough to account for the sum of the effect at 5° C. These results show that the alternation of temperature between $-5°$ and 5° C. had slight forcing action. This forcing action is equal to that obtained by keeping seeds in running water at 10° C. It is also evident that seeds ready to germinate should not be subjected to $-5°$ C.

The early changes taking place in seeds put to germinate at $0 \pm 1°$ C. were similar to those at 5° C. except for being retarded. At this temperature the increase in catalase activity was very much retarded, although it was over 3 times that gained by seeds stored at $-5°$ C. per unit time. These seeds were studied as to storage material, H^+ ion concentration, and permeability, and found in good condition. The *Juniperus* seeds not only after-ripened but germinated at $0 \pm 1°$ C., even though it required 5 or 6 months.

Moist seeds were placed at 5° C. for germination. At this temperature the catalase activity increased most rapidly. The physiological changes occurring in the seed at 5° C. were most rapid, and will be discussed in detail under changes preparatory to germination. This constant temperature of 5° C. also gave rise to by far the largest percentage of germination, and the most vigorous seedlings.

It is evident from these germination experiments that: (1) temperatures above 10° C. and below 0° C. are not favorable for after-ripening and germination; (2) no one of the forcing agents as used was of value in germination; (3) the inclosing structures do not inhibit germination; (4) but the inhibiting conditions are to be found in the endosperm and embryo. These facts indicate that the juniper seed has a dormant embryo that must go through

a series of fundamental changes before germination. Of the many points of attack that are suggested by these experiments two were chosen: (1) changes preparatory to germination, and (2) means of shortening the after-ripening period.

Changes preparatory to germination

These are the changes that occur in the seeds stored at 5° C. which prepare them for germination. As the embryo of the dry seed is morphologically complete, increases very little in size, and shows only the transformation of cell contents, these processes could be spoken of as "foregermination"; but as this term has not been used in this country these processes will be referred to as after-ripening. The first point studied was the imbibition of water.

TABLE X

SHOWING PERCENTAGE ABSORPTION OF WATER

(SEEDS DRIED AT 105° C. FOR MOISTURE DETERMINATION)

Material	Percentage water	Weight	Weight after submergence for hours indicated									Percentage water at maximum imbibition
			2	4	8	16	24	72	96	120	360	
Entire seed	7.00	2.0018	2.16	2.37	2.40	2.41	2.42	2.42	2.43	2.42	2.39	23.22
Endosperm and embryo coats off during imbibition	7.19	0.290	.....	.....	.....	.....	0.37	0.38	.....	0.37	.....	28.94
Endosperm and embryo coats on during imbibition	7.19	0.291	.....	.....	.....	.....	.....	.....	0.375	.....	.....	24.84

Table X shows that the seeds decreased slightly in weight after a few days, even when submerged in water. In examining the tables given by LAKON for the water absorption of seeds of *Pinus*, it was noted that he incidentally obtained similar results. To follow this more closely, seeds with coats on were placed on moist filter paper at 5° C., and at times samples were selected, coats removed, and the percentage of water in the seed, exclusive of coat, determined. Table XI gives these results and the percentage of water in the seedlings as well. It should be noted that the water content of the seed decreased gradually until germination, when there appeared a very marked increase up to the time of the developed seedling. This percentage of water seems to be related to the change in the water absorbing power of seed contents, and

not to changes in the permeability of the coat, as later experiments show.

Table XII gives the changes of H^+ ion concentration as P_H values for the endosperm and different parts of the embryo during storage at 5° C. The outer cells of the embryo and its hypocotyl were the first parts to show an increased H^+ ion concentration.

TABLE XI

PERCENTAGE OF WATER IN SEEDS TAKEN FROM COATS AFTER DIFFERENT PERIODS OF EXPOSURE TO 5° C.

Seeds	Percentage water
Dry	7.19
After 5 days at 5° C	24.84
After 15 days at 5° C	23.34
After 30 days at 5° C	23.09
After 60 days at 5° C	23.00
After 90 days at 5° C	23.21
After 100 days at 5° C. (coat splitting open)	52.64
After 130 days at 5° C. (seedlings 25 mm. long)	88.38

TABLE XII

H+ION CONCENTRATION OF SEEDS DURING AFTER-RIPENING*

Condition	Part of seed	P_H
Dry	Endosperm	4.4–6.0
Dry	Embryo	8.4–8.8
After 30 days at 5° C	Endosperm	4.6–5.2
After 30 days at 5° C	Embryo	6.8–7.6
After 60 days at 5° C	Endosperm	4.4–6.0
After 60 days at 5° C	Embryo	6.8–7.6
After 90 days at 5° C	Embryo hypocotyl	6.0–6.8
After 90 days at 5° C	Endosperm	4.4–5.2
After 90 days at 5° C	Embryo outer cells and hypocotyl	4.4–5.2
After 90 days at 5° C	Embryo inner cells	4.6–6.0

* These determinations were made with the Clark and Lubs indicators.

The embryo showed a marked increase of H^+ ion concentration during after-ripening, while the endosperm with P_H value of 4.4 (concentration of H^+ ions $\times N = 0.72 \times 10^{-4}$), being already acid, showed very little change. This may indicate that the embryo is the principal seat of dormancy. Table XIII gives the increase of titratable acid in the endosperm and embryo during after-ripening. The increased acid was determined by titrating with N/50 NaOH,

using phenolphthalein as the indicator. To show that this increased acid content is real the calculated dry weight of the seed material used is given.

The fat of the dry seeds is stored as very large globules, contrary to the statements of CZAPEK, which divide and become continually smaller as after-ripening goes on. Just preceding germination these fat globules, in the active growing cells, become reduced to microscopic size, although CZAPEK states that the microscopically divided fat of dry seeds collects into globules with early

TABLE XIII

INCREASE OF TITRATABLE ACID IN ENDOSPERM AND EMBRYO

Condition	No.	Dry weight in gm.	N/50 NaOH in cc.	Increased acid per unit volume of water
Dry	80	0.165	0.56	....
Dry	160	0.348	1.25	....
Dry	80	0.160	0.53	....
Dry	80	0.164	0.55	0.0254
After 15 days at 5° C	80	0.163	0.57	0.0989
After 30 days at 5° C	80	0.172	0.58	....
After 30 days at 5° C	80	0.172	0.60	0.0866
After 60 days at 5° C	80	0.169	0.70	....
After 60 days at 5° C	80	0.167	0.70	0.0685
After 95 days at 5° C	80	0.157	0.85	....
After 95 days at 5° C	80	0.163	0.90	0.0564
Open seeds	80	0.189	1.50	0.1426
Hypocotyl 2 mm	80	0.195	2.60	0.1615
Seedlings	80	0.210	8.00	....
Seedlings	80	0.204	8.00	0.1865

growth (7). This dispersion of the fatty material brings clearly into play surface tension, adsorption power, and many other forces resulting from the great increase of specific surface. Such a dispersion could lead to a more rapid digestion of the fats, thus materially aiding the transformation of fats to carbohydrates and the accumulation of energy for germination. The importance of making the fats more capable of transformation to carbohydrates should not be overlooked. It is also probable that this dispersion reaches a degree of division where it could aid in the translocation of fats as such. Thus highly dispersed fatty material would be carried through the cell walls at points of protoplasmic connection.

Early during the process of after-ripening there was a slight decrease in the fat content of the endosperm cells surrounding the embryo. The most rapid disappearance of fat occurred in the hypocotyl end of the endosperm at approximately the ninety-fifth day. This rapid decrease of fat was accompanied by an increase in the sugar content of the adjoining hypocotyl cells. This was the first noticeable increase of sugar during after-ripening. At this time the coat splits open, probably partly due to the increased osmotic pressure of the newly synthesized sugar. With these changes the first detectable starch was found. It increased very rapidly in these cells, until they seemed to be completely packed. Traces of starch appeared in the cotyledons and they soon became green, a point to be taken up later. Thus during the preparation for germination the stored fat was transformed into carbohydrates. Not all the fat is changed directly into carbohydrates. Under certain conditions it seems to be changed into forms more capable of translocation and used to synthesize other compounds, or even stored again. It seems that a large part of the food material of these seeds during after-ripening, germination, and the development of the seedling is translocated in this form.

Amino acids appear in both ungerminated (dry) and germinated seeds. Table XIV gives the amino acids found in these seeds, as well as a rough estimate of their quantities. The histidine in the endosperm was used up completely during the after-ripening.

Table XV gives the changes occurring in the proteins of *Juniperus* seeds during germination as indicated by color reaction. These results show that soluble proteins increased during after-ripening. It was also shown that the proteins were hydrolyzed during after-ripening by the determination of amino nitrogen and the formal titration. Table XVI gives the results of the VAN SLYKE determination for amino acids. This table shows that the 5 minute reaction period was too short, which indicates the presence of amino acids with other than α-amino groups. The arginine found would account for the increase under 30 minutes reaction. These figures prove that there was a marked hydrolysis of the proteins during after-ripening, as well as during germination and the development of

seedlings. As this protein digestion goes on, the number of free amino groups increases because of the splitting amino-carboxyl linkings. When hydrogen of the free amino group is replaced by

TABLE XIV

TESTS FOR AMINO ACIDS IN JUNIPER SEEDS

AMINO ACIDS	CRYSTALLIZATION	COLOR REACTIONS	AMOUNT OF AMINO ACIDS IN			
			Dry seeds		After-ripened seeds	
			Endosperm	Embryo	Endosperm	Embryo
Histidine...	+	Ehrlich's diazo	+++	+++	+	++
Tyrosine....	+	Ehrlich's diazo	+	+	++	++
Tyrosine....	+	Millons	+	+	++	++
Tyrosine....	+	Xanthroproteic	+	+	++	++
Cystine....	+	Sulphur reduction	+	+	+	+
Leucine....	+	....	+	+	++	++
Arginine....	+	....	+	+	++	++

TABLE XV

CHANGES IN STORED PROTEIN FOOD DURING GERMINATION

REACTIONS	DRY SEEDS			AFTER-RIPENED SEEDS		
	Endosperm	Embryo	Hypocotyl	Endosperm	Embryo	Hypocotyl
Biuret............	++	+++	+++	+	+	++
Millons...........	+++	+++	+++	++	++	+
Xanthroproteic.....	+++	++	++	+++	++	+
Berlin blue........	+	?	?	+	++	+++

TABLE XVI

INCREASE OF AMINO NITROGEN DURING AFTER-RIPENING AND GERMINATION

Condition of seed material	Time of reaction (min.)	N (cc.)	Temperature	Pressure (mm.)	Nitrogen obtained (mg.)	Amino acid as percentage of dry weight
Dry or resting..............	5	0.25	23.2	753.2	0.138	0.035
Coats bursted after 100 days at 5° C..................	5	0.50	24.7	750.8	0.274	0.270
Hypocotyl 3 mm. long or after 105 days at 5° C..........	5	0.59	24.5	751.0	0.324	0.275
Developed seedling or after 130 days at 5° C..........	5	1.41	24.5	751.3	0.775	0.921
Dry or resting..............	30	0.60	23.2	753.2	0.332	0.036
Coats bursted after 100 days at 5° C..................	30	0.84	24.2	750.7	0.462	0.279
Hypocotyl 3 mm. long or after 105 days at 5° C..........	30	1.17	24.5	750.7	0.642	0.280
Developed seedling or after 130 days at 5° C..........	30	1.97	25.2	750.7	1.078	0.935

methylene, the basicity becomes reduced; and the substituted acid can then be titrated with sodium hydrate as a measure of protein hydrolysis. Titrations made on a second lot of seeds according to the SORENSEN method gave results similar to the VAN SLYKE determinations.

The growth in these seeds occurring before germination is very meager. There is no morphological change in endosperm or embryo, although the latter increased slightly in length. After the appearance of sugar the hypocotyl exerts a forward pressure, separating the sides of the swelling cap which forces the coat open. At this moment the cap is under so much pressure that it is distorted, and a sharp angle is formed between its end and sides. The growth following this stage will be discussed later.

TABLE XVII

RESPIRATION OF SEEDS AT DIFFERENT PERIODS OF DEVELOPMENT AT 25° C. (7 CC. VOLUME)

Condition of seeds	No.	Green weight	Days	Percentage CO_2	Percentage CO_2+O_2	Percentage O_2 used	$\frac{CO_2}{O_2}$	Mgm. CO_2 per hour per gm.	Mgm. O_2 per hour per gm.
Dry	500	1.250	5	1.18	20.82	1.57	0.76	0.00098	0.0011
After 5 days at 5° C.	50	0.125	1	3.15	20.39	3.77	0.84	0.1311	0.1347
After 30 days at 5° C.	10	0.030	3	3.78	20.82	3.98	0.94	0.218	0.1976
After 60 days at 5° C.	10	0.027	3	3.80	20.70	3.90	0.97	0.2352	0.2151
After 90 days at 5° C.	10	0.028	3	3.80	20.68	3.90	0.97	0.2354	0.2075
After 100 days at 5° C.	10	0.028	3	4.10	20.00	6.00	0.68	0.2486	0.3192
After 130 days at 5° C.	10	0.099	1	9.30	20.56	9.74	0.95	0.4890	0.4398

Table XVII gives the results of the respiration experiments obtained by the use of the Bonnier and Mangin apparatus. There was a great increase in the respiratory intensity during the first 5 days and after the seeds split open. These are the periods when the seed increased in water content. There was a very slow increase in the respiratory intensity during after-ripening, even though the water content decreased. The respiration quotient increased very slightly during after-ripening, but decreased to a minimum at germination. Not only does this low respiratory quotient of 0.68 indicate the time of intense fat metabolism, but at this particular period it was found that the fats were being transformed into carbohydrates. It would be interesting to know this quotient at 5° C., as it would probably be much lower. After germination

the seedlings gradually attained the ratio 1:1. This rise in the respiratory quotient was probably due to the oxidation of carbohydrates and the more intense respiration of the seedlings.

Table XVIII gives the results of intramolecular respiration. The method used was that of NICOLAS (**26**). The point to be noted here is the low I/N ratio (the intramolecular or anaerobic respiration divided by the normal respiration) for the seedlings.

Peroxidase was more generally present than oxidase. Quantitative oxidase activity determinations were made with the Bunzel apparatus. These results showed that there was no appreciable increase of oxidase activity until after germination.

TABLE XVIII

INTRAMOLECULAR RESPIRATION OF JUNIPER SEEDS, NO. 10, AT 25° C. (7 CC. VOLUME)

Condition of seeds	Weight	Days	Percentage CO_2	I/N ratio
After 30 days at 5° C.	0.030	3	1.70	0.44
After 90 days at 5° C.	0.028	3	1.67	0.43
After 100 days at 5° C.	0.028	3	1.66	0.40
After 130 days at 5° C.	0.099	1	0.95	0.10

The results of catalase determinations are given in table IX, which gives the average of a great number of experiments. It was found that (1) when seeds were placed under ordinary germination conditions at 5° C. the increase of catalase activity gave a measure of the after-ripening; (2) the gain in catalase activity above that of air-dry seeds was greatest at 5° C. in a germinator; (3) the gain at the other temperatures was slow at best; and (4) seeds soon lose their catalase activity when in a germinator at temperatures above 25° C. The precautions used in the catalase determinations have been stated.

CROCKER (**6**) speaks of the rise in vigor of seeds, as shown by their resistance to fungal attack, during after-ripening. The juniper seed is protected against fungi before germination by the heavy lignin coat. It was found that juniper seeds which had not been after-ripened soon succumbed to fungal growths with the removal of the coats. After-ripened juniper seeds, however, when freed from the coats, withstood dense fungal growths.

Many such experiments indicate that the vigor and resistance of the seed to fungi increased greatly during the after-ripening process. These results prove that the juniper seed has a dormant embryo that goes through certain definite and well defined fundamental chemical and physical changes before germination can occur. Some changes occur also in the endosperm.

SHORTENING AFTER-RIPENING PERIOD AT 5° C.—The after-ripening period was shortened considerably by the constant temperature of 5° C., as has been shown, but attempts to shorten further this after-ripening period at 5° C. seemed to meet with difficulties. GUPPY'S (**15**) method of forcing seeds to germinate by placing the soft pre-resting seeds (caught before going into the rest period) at 20° C. was tested. None of these seeds germinated, and it is evident that the juniper seed must pass through a more or less definite rest and after-ripening period. This period was not shortened by the removal of the seed coats. ECKERSON (**10**) states that dilute acids greatly shorten the after-ripening period of the hawthorn. Dilutions of HCl between N/100 and N/3200 had no effect upon the juniper seed. Neither sugar, enzyme, nor vitamine solutions shortened this period. Hydrogen peroxide gave no results. In the treatment with different percentages of oxygen, it was found that the catalase activity increased slightly with increased oxygen pressure, and that the germination was retarded two months. Seeds were treated with different percentages of ether ranging from 0.002 to 6.000. As long as these seeds were under the influence of ether they showed a decrease in catalase activity proportional to the percentage of ether used. After atmospheric conditions were restored, all seeds recovered their catalase activity, but the after-ripening period was lengthened from 1 to 3 months depending on the low and higher percentages of ether. If the ether acted by decreasing the permeability, then it was evidently reversible, contrary to the work of OSTERHOUT (**27**). It is more probable, however, that the ether acted as a narcotic agent. This is also shown by the behavior of the seed. Carbon dioxide was used in concentrations ranging from 0.5 to 100 per cent with a six day exposure. The higher percentages increased the catalase activity and shortened slightly the after-ripening period. The action here

was probably due to increased acidulation in the presence of an abundance of CO_2 and H_2O which could favor the digestion of fats and germination (**25**). Desiccation and moistening again of seeds at about the forty-fifth day after being placed in the germinator shortens the after-ripening period from 5 to 10 days. This may be due to one of the following causes: (1) earlier after-ripening of the coats, as they are found to split off more readily when desiccated, (2) upsetting of the chemical equilibrium by the great extraction of water, or (3) the increase of H^+ ion concentrations.

Discussion

CATALASE.—The catalase activity, as has been noted by previous investigators (**1**, **6**), was found to bear some relation to respiration. Increased catalase activity accompanied the intense respiration of *Juniperus* seeds stored in high percentages of oxygen, as decreased

TABLE XIX

INCREASED CATALASE ACTIVITY WITH DEVELOPMENT (FIGURED PER UNIT DRY WEIGHT)

CONDITION OF SEEDS	OXYGEN IN CC. LIBERATED DURING		
	1 min.	5 min.	10 min.
Air dry	2.5	5.4	5.8
After 45 days at 5° C.	3.6	7.5	9.1
After 95 days at 5° C.	5.0	9.1	12.0
After 100 days at 5° C. (coats split)	5.3	12.2	14.4
After 130 days at 5° C. (seedlings)	10.5	23.1	28.5

catalase activity accompanied the low respiration of seeds stored in low percentages of oxgyen. With the intense respiration at high temperatures there was an increased catalase activity, even though the seeds did not after-ripen or germinate. The highest catalase activity and the most intense respiration per unit of dry weight was found in the seedling stage (cf. tables XIX and XVII). The desiccation of seeds to a slight extent, which makes for a rapid absorption of oxygen through the coat, increased the catalase activity. Desiccation to the extent of retarding respiration reduced the catalase activity. Table XX shows these results. Both the respiration and the catalase activity of seeds were reduced at will

by submerging them in water. Although increased catalase activity generally accompanied intense respiration, this relationship did not always hold, for when seeds were submerged a long time the catalase activity slowly increased, but there was no increase of respiration intensity. An examination of tables IX and XVII will show that the catalase gain was proportionally very much larger than the respiration gain during after-ripening. It will also be noted that the catalase gain was greatest at 5° C., where the respiration was low. It is evident, therefore, that there may be increased catalase activity without an increase of respiration.

TABLE XX

CATALASE ACTIVITY OF AFTER-RIPENED AND DESICCATED SEEDS, NO. 30 (CALCULATED DRY WEIGHT 0.0696)

Treatment	O_2 cc. liberated after 10 min.
Complete imbibition	33
Slight desiccation	38
Strong desiccation	32
Second imbibition	36

RATE AND PERCENTAGE OF GERMINATION.—Juniper seeds germinate most readily at the low temperature of 5° C. These seeds germinate, although very slowly, at 0±1° C. They also germinate at 10° C. Seeds after-ripened at 5° C. and then placed at 10° C. germinated slower than those left at 5° C. After-ripened seeds were thrown into a state of secondary dormancy by exposure to temperatures above 12° C. Their catalase activity gradually decreased and germination ceased. After being thrown into secondary dormancy, several weeks at 5° C. were required to after-ripen the seeds again. The seeds which sank in water gave between 75 and 80 per cent germination at 5° C.

GROWTH OF SEEDLING.—Table XXI gives the rate and extent of growth for seedlings exposed to the light or the dark at different temperatures. All seeds were germinated at 5° C. and then transferred to the different temperatures. The length of the extending hypocotyl at the time of transfer was 0–1 mm. The seedlings grew the longest and fastest at 25° C. At 30° C. they never attained

a normal length, while at $0 \pm 1^\circ$ C. there was a slow but definite growth. It is important to note that 15° C. seedlings developed first and appeared the most healthy and sturdy. These seedlings

TABLE XXI

EFFECT OF LIGHT AND TEMPERATURE ON RATE AND EXTENT OF GROWTH*

Temperature	Light	Length of hypocotyl in mm. from time of transfer					
		3 days	7 days	11 days	13 days	18 days	26 days
30° C...	Dark	1	5	7	12	18	20
25° C...	Dark	10	35	40	55	60	Seedling
15° C...	Dark	4	18	29	Seedling	....	....
10° C...	Dark	3	10	30	35	Seedling	....
10° C...	Light	3	11	29	30	Seedling	....
5° C...	Dark	2.5	4	12	15	26	35
$0 \pm 1^\circ$ C...	Dark	0	1	2	3	4	5
-5° C...	Dark	0	Killed	0	0	0	0

*Average of 50 trials.

at 15° C. also showed the earliest and greatest development of chlorophyll. Light did not seem to affect unusually the extent or rate of growth.

PIGMENTS.—Carbohydrates and temperature may condition chlorophyll development. The seedling was found to develop chlorophyll in total darkness. Thus the cotyledons become green long before they break out of the coat. Chlorophyll appeared first in the cotyledons and accompanied the formation of starch. This points to the conclusion that soluble carbohydrates are necessary for the formation of chlorophyll, the view advanced by PALLADIN (28). Table XXII gives the results of experiments planned to determine the effect of light and temperature on greening. This shows that light affects in no way the rate or apparent depth of greening. It also shows that at 30° C. and at $0 \pm 1^\circ$ C. chlorophyll did not develop. As the plastids were found to be in good condition, it was thought probable that a lack of building material was inhibiting chlorophyll development. Glucose cultures were made, therefore, but the seedlings again failed to develop chlorophyll. This indicates that a certain temperature is necessary for chlorophyll development, regardless of carbohydrate supply,

the maximum, optimum, and minimum temperatures for chlorophyll formation in the seedlings being represented by temperatures somewhat below 30, 15, and somewhat above 0° C.

Seedlings grown at 0± 1° C. developed anthocyanin, while those grown at 30° C. developed xanthophyll. When cultures at 0± 1° C. were supplied with glucose they developed more anthocyanin. The seedlings grown at 30° C. were made to develop anthocyanin by the addition of glucose. From the foregoing it appears that the seedlings form various pigments according to their reserve sugar

TABLE XXII

EFFECT OF LIGHT AND TEMPERATURE ON DEVELOPMENT OF CHLOROPHYLL*

Temperature	Light	Estimated percentage of color after transfer								
		1 day	4 days	6 days	8 days	11 days	13 days	18 days	26 days	50 days
30° C.	Light	0	0	0	0	0	0	0	0	0
25° C.	Light	5	25	50	50	50	65	......	......	......
15° C.	Light	5	25	50	75	75	100	......	......	......
10° C.	Light	5	25	50	75	......	......	100	......	......
10° C.	Dark	5	25	50	75	......	......	100	......	......
5° C.	Light	......	......	......	5	......	......	50	......	......
5° C.	Dark	......	......	......	5	......	......	50	......	......
0± 1° C.	Light	......	......	......	......	......	......	......	......	0
0± 1° C.	Dark	......	......	......	......	......	......	......	......	0

*Average of 50 trials.

supply. Seedlings with little sugar tend to develop xanthophyll, those with more sugar chlorophyll, and those with an abundance of sugar anthocyanin.

PRACTICAL APPLICATION.—The foregoing experiments make it possible to devise an outline for the practical production of juniper plants. This should be of interest to growers, since it has furnished a means of increasing many fold the percentage of germination and of developed seedlings. After collection, the seeds are freed from the berries, sorted, and sterilized as has been described. The seeds are then put into Petri dishes or covered flat vessels on filter paper supported by wet cotton. These vessels of seeds are kept at a constant temperature of about 5° C. (41° F.) for after-ripening, which takes about 100 days. This after-ripening period can be shortened 10 days by drying slightly and moistening again the seeds at about the forty-fifth day. When the coats have split

open and the hypocotyls are $\frac{1}{8}$ in. long, the seedlings are transferred to pans or beds of leaf mold and sand kept at 15° C. (60° F.). In no case should ungerminated seeds (seeds that have not split open and developed a short hypocotyl) be transferred from the germinator at 5° C. (41° F.). The germinated seeds, after being transferred to beds or pans, should be protected by glass plates and paper for the first few days.

Although these seeds have been germinating during every month of the year, advantage can be taken of the temperature conditions by placing them in the germinator about January. The importance of this after-ripening and germination at 5° C. cannot be overemphasized.

Summary

1. The germination of non-after-ripened juniper seeds under ordinary conditions is very low, amounting to 1 per cent.

2. These seeds are protected by a semipermeable and thick coat which makes up 75 per cent by weight of the entire seed. Acids enter very slowly, while bases, silver and mercury salts, enter rapidly. While the coat serves as a protection against fungal attack and prevents water-imbibed seeds from expanding and rupturing the tissues before after-ripening is accomplished, it takes little or no part in the dormancy or after-ripening of the seed.

3. Food material in the resting seed is stored in the form of fats and proteins, with traces of glucose but no starch. The resting seed endosperm has a P_H value of about 5, while that of the embryo is about 8.

4. Although some forcing agents changed the respiration and catalase activity of seeds, it was not possible to force the germination of non-after-ripening juniper seeds by high temperature, alternating temperature, wounding, warm bath, dry air, removal of coats, treatment with hydrogen peroxide, mercuric chloride, ether, carbon dioxide, oxygen, light, soil, dilute acids, dilute bases, nitrates, suphates, or strong acids.

5. Freezing and thawing as such has no forcing action on the germination of juniper seeds, neither does it hasten after-ripening. Freezing and thawing produces marked chemical changes in this

seed, but these changes, as has been outlined, are quite different from those occurring during after-ripening. Seeds ready to germinate (after the coat is cracked and their water content increased to 52 per cent) are killed by an exposure to $-5°$ C.

6. The juniper seed has a dormant embryo that must after-ripen before germination. After-ripening occurs at temperatures between $0 \pm 1°$ C. and 10° C., although fastest at about 5° C.

7. The changes that accompany after-ripening of the juniper seed at 5° C. were found to be as follows: (1) rather rapid and complete imbibition, followed by a steady slow decrease in water content during after-ripening or until near germination; (2) increased H^+ ion concentration, especially of the embryo; (3) an increment of titratable acid; (4) a steady and enormous increase in the degree of dispersion of the stored fat; (5) decrease in the amount of stored fat and protein, with an increase of sugar content and the first appearance of starch; (6) the translocation of food in the form of fat or fatty acids from endosperm to embryo; (7) a seven-fold increase in the amino acid content, and a complete disappearance of histidine from the endosperm; (8) an increase of soluble proteins, with a marked hydrolysis of the stored proteins: (9) slight growth of embryo; (10) very slight increase of the respiration intensity; (11) increased respiratory quotient; (12) decreased intramolecular respiration; (13) a doubling of the catalase activity; and (14) the rise in vigor of seeds as shown by their resistance to fungal attack.

8. In conjunction with after-ripening at 5° C., desiccation seems to be the only promising means of shortening this after-ripening period.

9. The time at which the hypocotyl breaks through the nucellus was fixed as the end of after-ripening and the beginning of germination.

10. Neither the resting nor the after-ripened juniper seeds yield more than about 1 per cent germination at temperatures above 15° C. Seeds after-ripened at 5° C., then placed at 10° C., germinate slower than those left at 5° C. When after-ripened seeds are transferred from 5° C. to temperatures above 15° C. they are thrown into a state of secondary dormancy. Hence these seeds require a low temperature for germination as well as for after-ripening, and therefore no seed should be transferred to

higher temperatures until germination has started. If these seeds are given sufficient time they will germinate, even at $0 \pm 1°$ C.

11. Subsequent to after-ripening and germination at 5° C., the best temperature for seedling development is 15° C.

12. The development of chlorophyll in the juniper seed and seedling was found to be independent of light, but conditioned by the temperature range. Seedlings grown at temperatures of $0 \pm 1°$ C. or 30° C. never developed chlorophyll. Anthocyanin development in seedlings seems to depend upon relative temperature and carbohydrate supply.

13. A more complete chemical analysis of these seeds at different stages of development will be given in a later paper.

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